

Editorial

Non-CpG Methylation Revised

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Abstract: Textbook and scientific papers addressing DNA methylation usually still cite “DNA methylation occurs at CpG cytosines”. Methylation at cytosines outside the CpG nucleotide, the so-called “non-CpG methylation”, is usually considered a minor and not biologically relevant process. However, the technical improvements and additional studies in epigenetics have demonstrated that non-CpG methylation is present with frequency higher than previously thought and retains biological activity, potentially relevant to the understanding and the treatment of human diseases.

Keywords: DNA methylation; non-CpG methylation; DNMT3; bisulfite assay; neuroepigenetics

1. Introduction

Revising, and even rejecting, its own laws and dogmas is part of the honest and necessary process of scientific knowledge. This is the natural result of disciplines in which technical advances constantly challenge former theories as new findings are produced. In biology, one of the most typical examples of this process has been the revision of the “central dogma” [1] and of the unidirectionality of genetic information, which have been revised after the production of evidence of reverse transcription mechanisms. “Epigenetic science”, as part of the bio-medical science, is no exception and is therefore subject to continuous revision and adjustment. This is the case of the new perspective suggested by the increasing evidence that non-CpG methylation of DNA is a discrete event and retains its functional role [2–5].

Until the last decade, it was widely reported that the cytosines within the CpG dinucleotides were the primary, if not exclusive, site for DNA methylation in mammals. Although documented for many years, methylation of non-CpG sites (i.e., cytosines within the dinucleotides CpC, CpA, and CpT) was mostly found in plants and prokaryotes, whereas in mammals, it was classified as transient or limited to specific cell types. The majority of the studies referred to non-CpG methylation occurring only in embryonic tissues, stem cells, and oocytes [6,7]. The first evidence of systematic non-CpG methylation highlighted the relatively low frequency of this modification, usually calculated to be around 15–25% of total methylation in embryonic stem cells (ESCs) and induced pluripotent SCs (iPSCs) and circumscribed to gene bodies [8,9]. Non-CpG methylation in gene bodies was also functionally associated with gene expression [10].

After the bisulfite assay [11] gained popularity and became the gold standard for the study of DNA methylation, and was combined with different revelation methods, the evidence that non-CpG methylation has become more widespread than previously thought and can no longer be ignored [12–17]. Since its very first application, the methylation assay based on bisulfite modification showed the potential to disclose the discrete presence of non-CpG methylation in mammal DNA [18].

One possible cause of the tardy recognition of the extent of non-CpG methylation extent has been suggested by studies performed in my laboratory. After having observed unexpected non-CpG methylation associated with gene expression regulation in mouse myoblasts [19], we hypothesized that

other studies could have failed to observe it due to a technical issue in the bisulfite assay. We therefore demonstrated that non-CpG methylation underestimation was due to a technical bias due to the large use of software-designed primers in the polymerase chain reaction (PCR) step following the bisulfite modification of genomic DNA. These primers are usually designed with mutated bases at cytosines, assuming that non-CpG sites are not methylated (according to the former dogma of the DNA methylation). However, if discrete non-CpG methylation is present, the DNA is not modified by bisulfite and the target sequences cannot be recognized by the primers, resulting in selective amplification of DNA with low non-CpG methylation. Conversely, and according to the original technique, we proposed designing the primers considering the uncertainty of non-CpG methylation status, considering the presence of degenerated bases, in order to accommodate both methylated and non-methylated cytosines [20].

Due to technical advances and, in particular, unbiased epigenetic approaches, we can now state that non-CpG methylation occurs at higher frequencies than previously expected, ranging from 25% to 35% in mice and humans, particularly in adult mammalian somatic cells including the adult mammalian brain, skeletal muscle, and hematopoietic cells [2,21,22]. In parallel to this new awareness of the non-CpG methylation frequency in differentiated tissues and adult tissues in mammals, recent results increasingly highlight its functional role [23–27] as well as the identity of several genes that are functionally regulated by non-CpG methylation [28–32].

Non-CpG methylation seems to be functionally associated, in particular, with gene regulation in the brain and in the nervous system. Once established during embryonic neurogenesis, non-CpG methylation is conserved during adult life and can account for 53% of total 5-mC, representing the main form of neuronal DNA methylation [33]. Evidence of differential non-CpG methylation correlated to brain pathology and brain aging has been collected both through gene-specific and genome-wide analyses [4,5,34–37]. My laboratory further contributed to this field, taking advantage of the unbiased approach described above, showing that differential non-CpG methylation is associated with the modulated expression of specific genes in the brain of human patients with Alzheimer's disease and Tuberos Sclerosis [38–41].

The data demonstrating that non-CpG methylation is not restricted to embryonic or pluripotent cells but is, on the contrary, widely present in adult tissue and particularly in tissues with low cell turnover such as the brain, are now abundant and consistent. The evidence that the epigenetic mark retains its functional role in gene expression modulation and that it is potentially associated to human diseases is continually increasing. Therefore, the time has come to revise the epigenetic dogma that DNA methylation occurs at CpG sites in mammals. The study of methylation at non-CpG sites could result in new perspectives for the challenge presented by the understanding and the treatment of several brain diseases.

Conflicts of Interest: The author declares no conflict of interest.

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